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Presence of β-Lactamase Gene TEM-1 DNA Sequence in Commercial *Taq* DNA Polymerase

The development and spread of expanded-spectrum β-lactamases (ESBLs) that cause resistance to β-lactam antibiotics has contributed to great concern worldwide. Most ESBLs are derived from TEM and SHV β-lactamases by point mutations within the bla_{TEM} and bla_{SHV} genes, giving rise to extended-spectrum drug resistance (2, 3). The standard method for determining the specific ESBL gene for the more than 90 TEM-type and the more than 25 SHV-type ESBLs is PCR followed by nucleotide sequencing (2).

We routinely applied the standard method to confirm that ESBL genes were present in clinical strains of *Enterobacteriaceae* (6). To avoid cross-contamination, we used separate rooms for sample preparation, PCR assembly, and agarose gel analysis. Recently, we noticed that the negative (water) controls used in PCR amplification for bla_{TEM} genes produced positive results; this did not occur when the bla_{SHV} gene was targeted. The PCR product was of the predicted size, and nucleotide sequencing revealed that it was the bla_{TEM-1} gene. After systematic analysis of pipette tips, microcentrifuge tubes, and reagents for PCR, we found that the *Taq* DNA polymerase was the source of contamination. As shown in Fig. 1, *Taq* DNA polymerase from manufacturer A produced a strong signal, but that from manufacturer B did not.

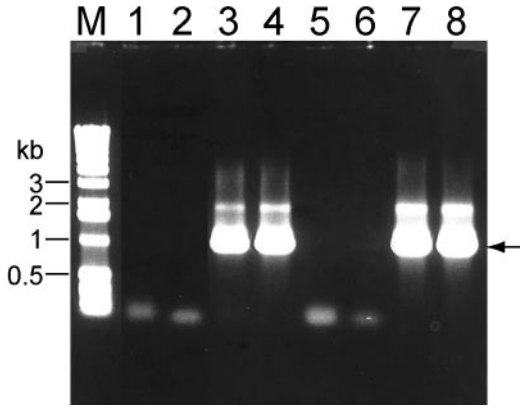

FIG. 1. PCR products amplified from negative (water) controls with primers targeting the bla_{TEM} gene. The product was run on a 1% agarose gel with 0.5× Tris-acetate-EDTA buffer. All reactions were performed in duplicate. Lane M, DNA size marker; lanes 1 and 2, PCR buffer from manufacturer B with *Taq* DNA polymerase from manufacturer B; lanes 3 and 4, PCR buffer from manufacturer B with *Taq* DNA polymerase from manufacturer A; lanes 5 and 6, PCR buffer from manufacturer A with *Taq* DNA polymerase from manufacturer B; lanes 7 and 8, PCR buffer from manufacturer A with *Taq* DNA polymerase from manufacturer A. The arrow indicates the amplified bla_{TEM-1} gene fragment.

PCR is widely used to detect specific DNA sequences for purposes of microbial identification, clinical diagnosis, and basic research. Because the method is extremely sensitive, a small amount of contaminating DNA can be a serious problem. *Taq* DNA polymerase is often expressed as a recombinant protein in *Escherichia coli*. For studies involving gene cloning and protein expression in *E. coli*, the bla_{TEM-1} gene has been the most commonly used selective marker for expression vectors that are generally present in multiple copies (9). It is likely that during *Taq* DNA polymerase purification, the DNA containing the bla_{TEM-1} gene was not completely removed. This failure may not be a rare occurrence, but the contamination would be detected only if primers specific for bla_{TEM} gene were used.

Several reports have documented the presence of exogenous DNA in commercial *Taq* DNA polymerases (1, 4, 5, 7, 8, 10). Sources of the contaminating DNA have ranged from bacteria (1, 4) and phage-like DNA (7) to both prokaryotes and eukaryotes (10); in other studies, it was determined that the contaminating DNA was not from *E. coli* or *Thermus aquaticus* (5, 8). In all of these previous reports, PCR amplification was performed with universal primers for the highly-conserved 16S rRNA gene, whereas in the present study, amplification was done with primers targeting the bla_{TEM} gene. Nevertheless, investigators, especially those who work on TEM-type ESBLs, should be aware of the possibility that *Taq* DNA polymerase is contaminated with the bla_{TEM-1} gene.

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